

IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: The Survival of Bacteria on Alpha SanoProtex at 65% RH.

CLIENT: Akzo Nobel Decorative Paints Continental Europe

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1 Introduction

This report summarises a study performed to assess the survival of bacteria at 65% relative humidity on surfaces coated with emulsion paints formulated either with or without antimicrobial agents. Test panels coated with the paints as applied were inoculated with either methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Streptococcus pneumonia* or *Enterococcus hirae* and then incubated at 65% relative humidity. The survival of these bacteria on the surfaces was then measured over a 1 day period by measuring total viable count (as colony forming units).

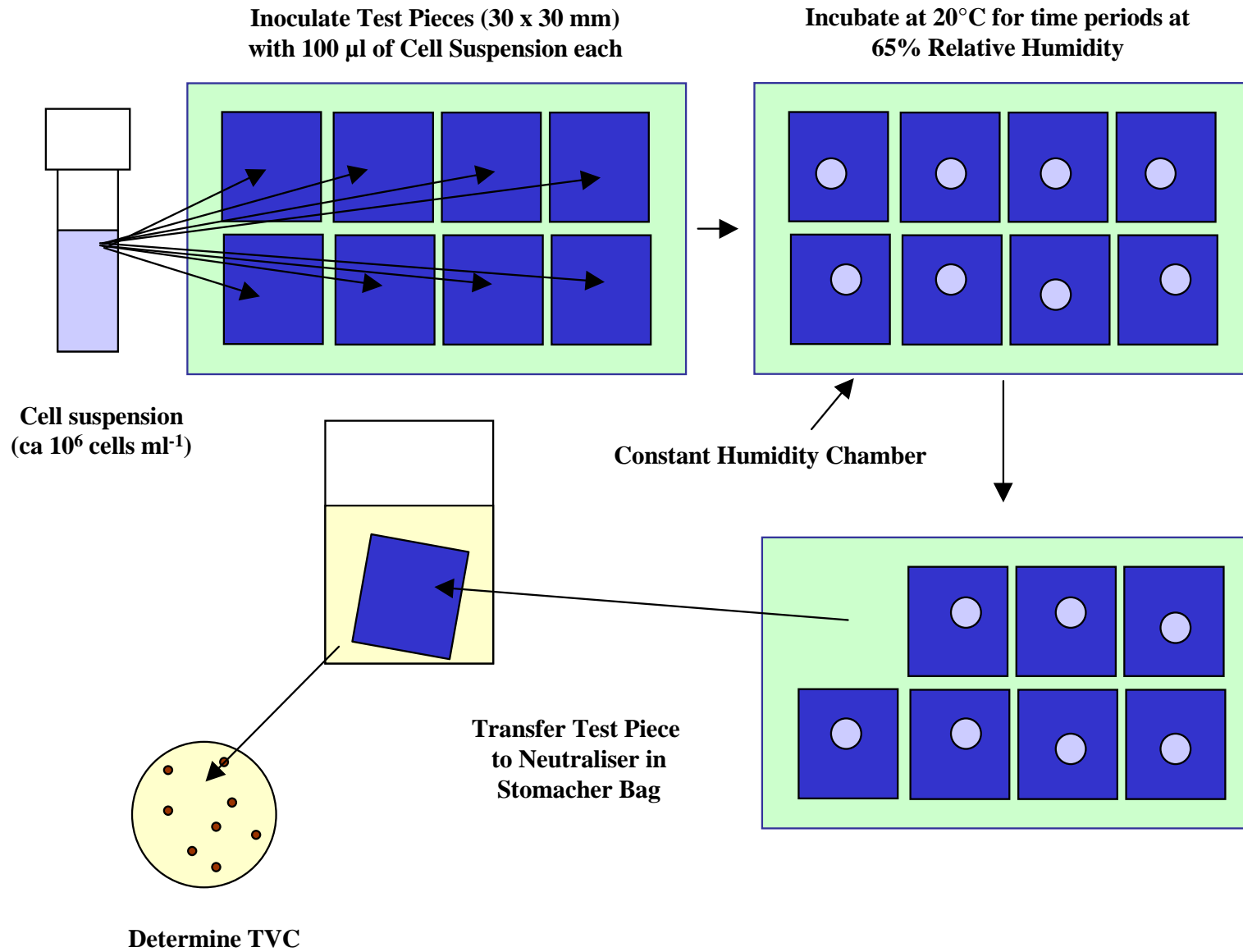
2 Test Materials

Replicate flexible test panels (Leneta scrub resistance test panels) which had been coated by block spreader (250 µm wet film thickness) with either Alpha SanoProtex or a conventional emulsion paint were supplied by Akzo Nobel Decorative Paints Continental Europe. On receipt at IMSL, all samples were held in the dark at 20°C prior to testing. Prior to inoculation, the individual test panels were cut into sections (each 40 mm x 40 mm) to provide replicate sub-samples of each coating type. Groups of sub-samples were then placed into chambers maintained at a constant humidity of 65% RH. The sub-samples were then allowed to equilibrate for 24 hours at 20°C.

3 Methods

Replicate aliquots (each 100 µl) of a log phase cell suspension of either MRSA (NCTC 11939, 6.7×10^6 cells ml⁻¹), *Pseudomonas aeruginosa* (ATCC 15442, 7.8×10^6 cells ml⁻¹), *Escherichia coli* (ATCC 8739, 6.3×10^6 cells ml⁻¹), *Acinetobacter baumannii* (ATCC 19606, 3.1×10^6 cells ml⁻¹), *Streptococcus pneumonia* (ATCC 6303, 3.0×10^6 cells ml⁻¹) or *Enterococcus hirae* (ATCC 10541, 6.1×10^6 cells ml⁻¹) in sterile distilled water were placed on the surface of each of the test sub-samples in the humidity chambers. The chambers were then incubated for up to 24 Hours at 20°C. Three sub-samples selected at random were removed from each of the chambers after 0, 6 Hours, 12 Hours and 24 Hours. The sub-samples were then processed using the method and neutraliser described in JIS Z 2801 (see Ref 1 and Figure 1). The viable population present in the suspension resulting from this process was enumerated by spiral dilution or pour plate technique using Trypcase Soya Agar (incubated at 37°C for 24 hours), Nutrient agar (incubated at 30°C for 24 hours) or Columbia Agar plus 5% Horse blood (incubated at 30°C under micro-aerophilic conditions for 24 hours) as appropriate and using a pour plate technique with molten Trypcase Soya Agar (incubated at 37°C for 24 hours).

Figure 1: Schematic Diagram of Method



4 Results / Discussion

The results for the survival of the organisms on the test coatings are shown in Tables 1 - 6 and Figure 2 - 3. In the tables, the data is expressed as colony forming units (CFU) cm⁻² and in the figures as the base 10 logarithm of this data. The theoretical limit of detection for the method was 1.25 CFU cm⁻². The statistical analysis of the data is shown in Tables 7 - 12.

Table 1: Survival of *E coli* on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	3.1 x 10 ⁵	1.6 x 10 ⁵	8.3 x 10 ⁴	1.1 x 10 ⁵
Alpha SanoProtex	3.1 x 10 ⁵	1.2 x 10 ⁵	3.9 x 10 ⁴	< 1.25

Table 2: Survival of MRSA on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	3.3 x 10 ⁵	1.5 x 10 ⁵	1.2 x 10 ⁵	5.2 x 10 ³
Alpha SanoProtex	3.3 x 10 ⁵	9.8 x 10 ⁴	6.0 x 10 ³	5.2 x 10 ⁰

Table 3: Survival of *Ps aeruginosa* on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	3.9 x 10 ⁵	9.2 x 10 ⁴	4.5 x 10 ⁴	< 1.25
Alpha SanoProtex	3.9 x 10 ⁵	4.0 x 10 ⁴	< 1.25	< 1.25

Table 4: Survival of *Acinetobacter baumannii* on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	1.5 x 10 ⁵	1.4 x 10 ⁵	5.2 x 10 ⁴	< 1.25
Alpha SanoProtex	1.5 x 10 ⁵	1.4 x 10 ⁴	6.8 x 10 ¹	< 1.25

Table 5: Survival of *Streptococcus pneumoniae* on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	1.5×10^5	7.6×10^4	5.1×10^4	1.8×10^3
Alpha SanoProtex	1.5×10^5	8.2×10^4	7.4×10^3	1.0×10^3

Table 6: Survival of *Enterococcus hirae* on Coated Surfaces at 65% Relative Humidity

Sample	Exposure Time			
	0	6 Hours	12 Hours	24 Hours
Conventional paint	3.1×10^5	2.1×10^5	1.6×10^5	1.1×10^5
Alpha SanoProtex	3.1×10^5	1.7×10^5	9.9×10^4	2.4×10^3

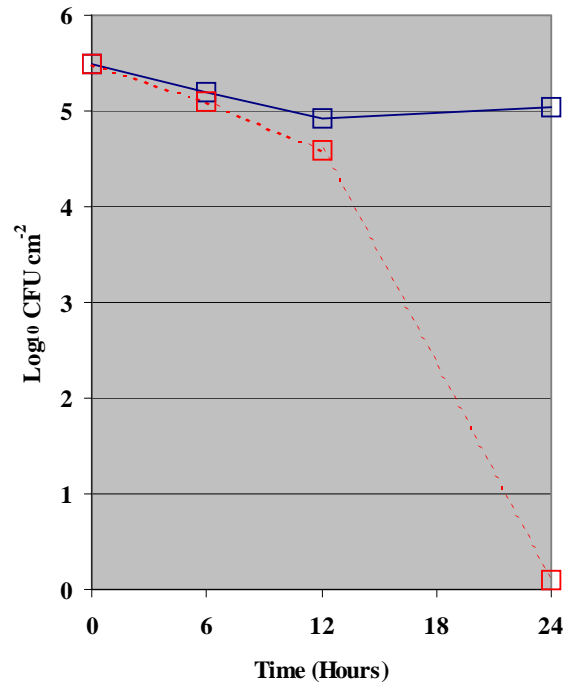
It can be seen from the results above that the populations of *E coli*, MRSA, *E hirae* and *Str pneumoniae* remained viable on the surface of the conventional paint for the duration of the 24 hour contact period. In comparison, the populations of *Pseudomonas aeruginosa* and *A baumannii* remained viable for the initial 12 hour contact period and then declined to below the limit of detection following the subsequent 12 hours of the exposure interval.

The populations of *E coli*, MRSA, *E hirae*, *Str pneumoniae*, *Ps aeruginosa* and *A baumannii* exposed to the surface of Alpha SanoProtex declined at a faster rate than the populations exposed to the conventional paint. The resulting differences from the conventional paint were statistically significant after 12 hours for the populations of MRSA, *Pseudomonas aeruginosa* and *A baumannii* and after 24 hours for the populations of *E coli*, *Str pneumoniae* and *E hirae*.

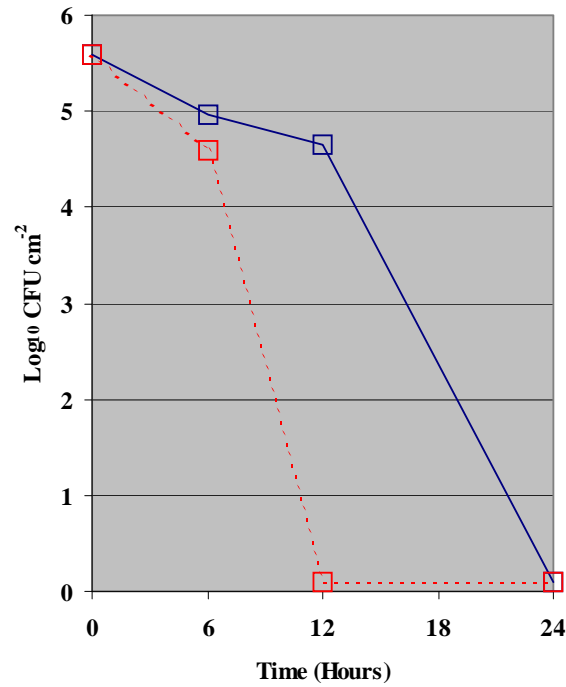
In general, the exposure of the microbial populations tested as splashes of contaminated liquid to Alpha SanoProtex resulted in a faster rate of decline than when exposed to the conventional paint.

Figure 2: Survival of *E coli*, *Ps aeruginosa* and *A baumannii* at 65% Relative Humidity on Coated Surfaces

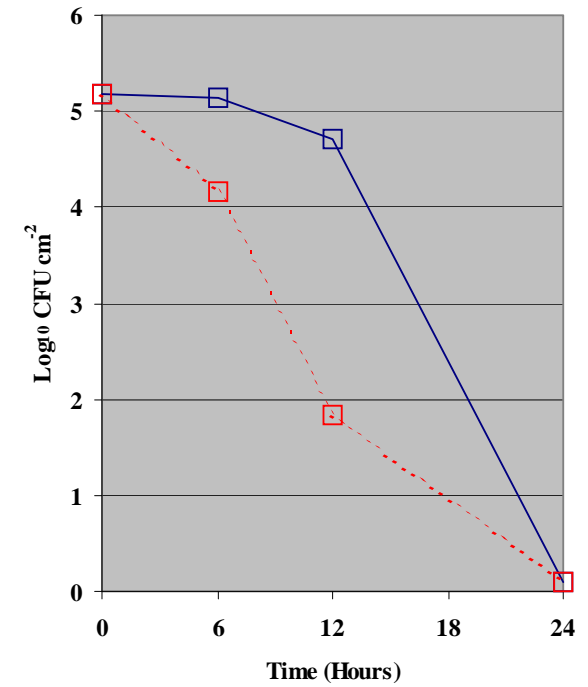
E coli



Ps aeruginosa



A baumannii



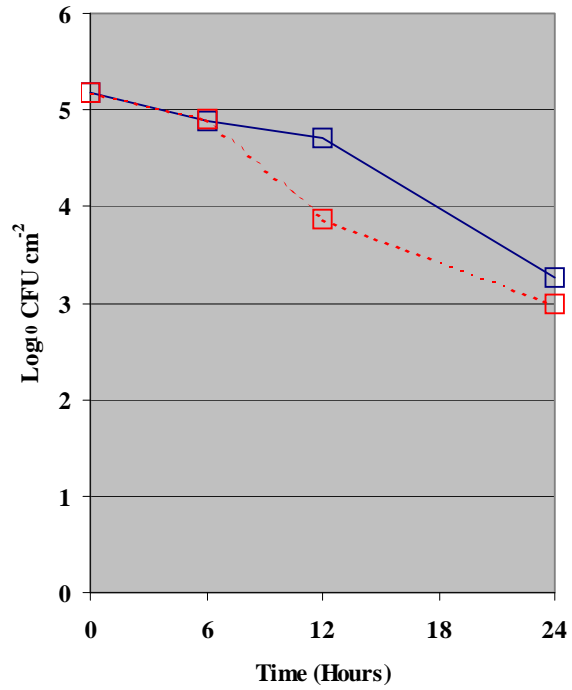
—□— Conventional paint - - - □ - - - Alpha SanoProtex

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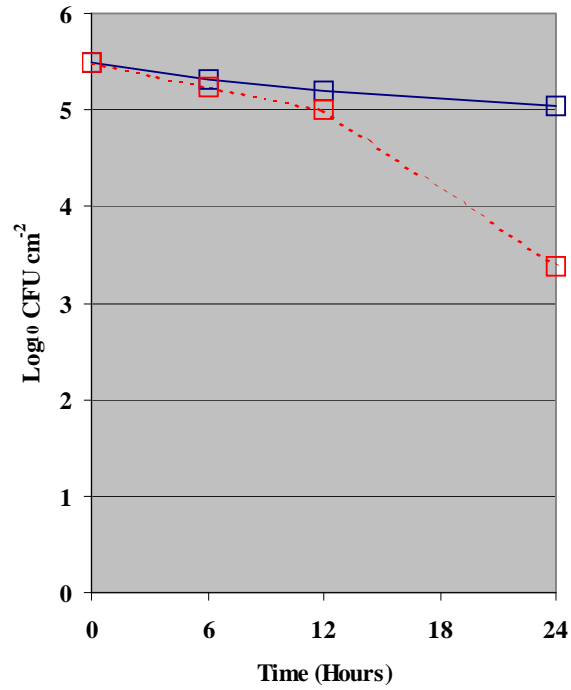
Figure 3: Survival of MRSA, *E hirae* and *Str pneumoniae* at 65% Relative Humidity on Coated Surfaces

Strep pneumoniae



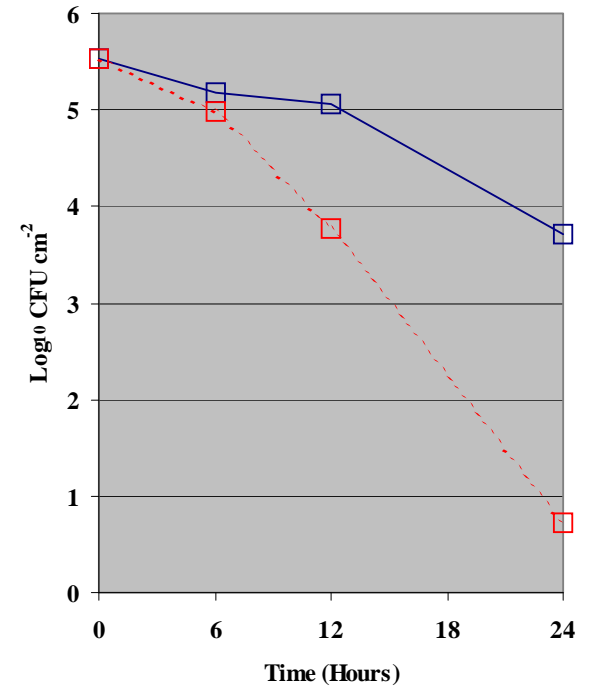
—□— Conventional paint - - - □ - - - Alpha SanoProtex

E hirae



—□— Conventional paint - - - □ - - - Alpha SanoProtex

MRSA



—□— Conventional paint - - - □ - - - Alpha SanoProtex

Table 7: Statistical Analysis of Effects Observed Against *E coli* (ANOVA) after 12 and 24 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex.	3	4.590	0.1902	0.1188	0.329
Conventional paint	3	4.920	0.0437	0.1188	0.076

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	0.227	3	0.076	1.79	0.2272
Residual	0.339	8	0.042		
Total	0.566	11			

LSD Contrast	Difference	95% CI
Alpha SanoProtex v Conventional paint	-0.330	-0.717 to 0.057

Log ₁₀ CFU cm ⁻² by Treatment after 24 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	0.097	0.0000	0.0757	0.000
Conventional paint	3	5.044	0.1335	0.0757	0.231

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	54.111	3	18.037	1049.10	< 0.0001
Residual	0.138	8	0.017		
Total	54.249	11			

LSD Contrast	Difference	95% CI	
Alpha SanoProtex v Conventional paint	-4.947	-5.194 to -4.700	(significant)

Table 8: Statistical Analysis of Effects Observed Against MRSA (ANOVA) after 12 and 24 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	3.777	0.1165	0.0959	0.202
Conventional paint	3	5.071	0.0999	0.0959	0.173

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	6.548	3	2.183	79.05	< 0.0001
Residual	0.221	8	0.028		
Total	6.768	11			

LSD Contrast	Difference	95% CI	
Alpha SanoProtex v Conventional paint	-1.294	-1.607 to -0.981	(significant)

Log ₁₀ CFU cm ⁻² by Treatment after 24 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	0.716	0.6191	0.3980	1.072
Conventional paint	3	3.715	0.0915	0.3980	0.158

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	16.887	3	5.629	11.84	0.0026
Residual	3.802	8	0.475		
Total	20.690	11			

LSD Contrast	Difference	95% CI	
Alpha SanoProtex v Conventional paint	-2.999	-4.297 to -1.701	(significant)

Table 9: Statistical Analysis of Effects Observed Against *Ps aeruginosa* (ANOVA) after 12 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	0.097	0.0000	0.0901	0.000
Conventional paint	3	4.651	0.0714	0.0901	0.124
Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	46.109	3	15.370	631.78	< 0.0001
Residual	0.195	8	0.024		
Total	46.304	11			
LSD					
Contrast	Difference	95% CI			
Alpha SanoProtex v Conventional paint	-4.554	-4.848 to -4.261		(significant)	

Table 10: Statistical Analysis of Effects Observed Against *A baumannii* (ANOVA) after 12 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	1.833	0.0717	0.1332	0.124
Conventional paint	3	4.712	0.1059	0.1332	0.183
Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	20.752	3	6.917	130.00	< 0.0001
Residual	0.426	8	0.053		
Total	21.178	11			
LSD					
Contrast	Difference	95% CI			
Alpha SanoProtex v Conventional paint	-2.879	-3.314 to -2.445		(significant)	

Table 11: Statistical Analysis of Effects Observed Against *Str pneumoniae* (ANOVA) after 12 and 24 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	3.866	0.9386	0.4859	1.626
Conventional paint	3	4.705	0.0812	0.4859	0.141
Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	1.099	3	0.366	0.52	0.6819
Residual	5.665	8	0.708		
Total	6.765	11			

LSD

Contrast	Difference	95% CI
Alpha SanoProtex v Conventional paint	-0.839	-2.424 to 0.745

Log ₁₀ CFU cm ⁻² by Treatment after 24 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	2.998	0.1007	0.0655	0.174
Conventional paint	3	3.261	0.0349	0.0655	0.060
Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	0.510	3	0.170	13.22	0.0018
Residual	0.103	8	0.013		
Total	0.613	11			

LSD

Contrast	Difference	95% CI	
Alpha SanoProtex v Conventional paint	-0.263	-0.476 to -0.049	(significant)

Table 12: Statistical Analysis of Effects Observed Against *E hirae* (ANOVA) after 12 and 24 Hours Contact

Log ₁₀ CFU cm ⁻² by Treatment after 12 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	4.997	0.1255	0.2104	0.217
Conventional paint	3	5.192	0.0153	0.2104	0.027

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	0.298	3	0.099	0.75	0.5530
Residual	1.063	8	0.133		
Total	1.361	11			

LSD Contrast	Difference	95% CI
Alpha SanoProtex v Conventional paint	-0.195	-0.881 to 0.491

Log ₁₀ CFU cm ⁻² by Treatment after 24 hours contact	n	Mean	SE	Pooled SE	SD
Alpha SanoProtex	3	3.385	0.8071	0.4613	1.398
Conventional paint	3	5.049	0.1417	0.4613	0.245

Source of variation	Sum squares	DF	Mean square	F statistic	p
Treatment	14.238	3	4.746	7.43	0.0106
Residual	5.107	8	0.638		
Total	19.345	11			

LSD Contrast	Difference	95% CI	
Alpha SanoProtex v Conventional paint	-1.664	-3.168 to -0.160	(significant)

5 Raw Data

The raw data for this study will be held in file IMSL2009/04/009 in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 6 years from the date of this report unless other specific instructions are given.

6 Exclusion of Liability

The contents of this report are subject to the standard terms and conditions of IMSL as displayed on the reverse of the invoice. Specific attention is drawn to Section 10 restated below.

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